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A COMPUTER SYSTEM FOR COMBINED NEURONAL MAPPING AND MORPHOMETRY--ETC(U)
JUL 80 C A CURCIO, K R SLOAN N00014-78-C-0164
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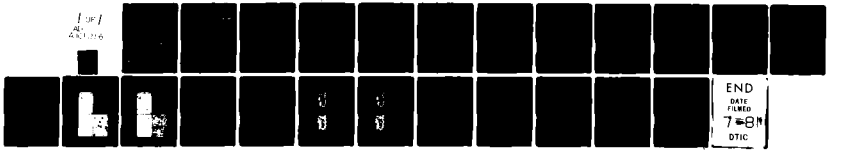

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(6) A Computer System for Combined Neuronal Mapping and Morphometry,

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Abstract

A computer system has been developed which allows both mapping and planimetry of histological preparations. Data consist of cytoarchitectonic boundaries defined in low power micrographs of thick sections, and nuclear and vascular profiles defined in high power micrographs of thin sections taken from the original thick tissue, and landmarks common to both. Data from these different preparations and magnifications are entered into the computer by a digitizing tablet and are stored as points in a real-world coordinate system, along with profile labels, landmarks for alignment purposes, and section depth. Subsequent programs determine parameters such as area and perimeter for each profile, as well as its geometric center and relationship to the cytoarchitectonic boundary. This mapping parameter allows morphometric analysis of profiles as a function of their position. Other programs allow display and manipulation of data in three dimensions, cell counts, and stereology.

The preparation of this paper was supported in part by NIH Training Grant GM07136, NIH Grant AG1121, NIH Grant NS15283, and the Defense Advanced Research Projects Agency, monitored by the ONR, under Contract (15) N00014-78-C-0164, PHS-5-0[illegible]

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Introduction

The diversity and individuality of neuronal forms present challenging problems for the quantitative morphologist. Increasingly, investigators are turning to computerized methods for analysis of structure (for review, see [Woolsey and Dierker, 1979; Lindsay, 1977]). For example, computer systems now exist in which dendritic or axonal arbors may be reconstructed from thick or serial thin sections for improved visualization, quantification, and modeling.

Less elaborate systems have been designed for topographical analysis of histological preparations. These systems have in common an X-Y coordinate system defined by the microscope stage into which tissue features are placed as single points, either manually or in a semi-automated fashion. Examples of this type of system are the mapping of silver grains [Wann et al., 1974] and degenerating axon terminals [Forbes and Perry, 1979].

Finally, there are systems which perform planimetric functions, such as area and perimeter, on profiles seen in tissue preparations (e.g., [Cowan and Wann, 1973]). These systems consist of a digitizing tablet, onto which profiles are traced either directly from the microscope or from photographs or drawings, and a minicomputer, which performs planimetry and simple statistical analyses.

We have designed a computer system which combines the latter two functions, i.e., mapping of profiles and calculation of descriptive parameters. This system consists of a digitizing tablet and personal computer connected to a large time-sharing computer. It allows mapping and quantitative description of multiple profile classes, selection of profiles for analysis by location, calculation of cell density, and display. In this report, we present a description of this system and its application to cortical cell counts.

Concrete Example

The system described in this paper is general. However, its development was motivated by a particular problem. In order to add concreteness to our discussion, we briefly describe the specific application here.

Neuron loss in the cerebral cortex is a hallmark of human senescence [Brody, 1955; Brody, 1970; Henderson et al., 1979]. A problem inherent in these studies is the relationship of cell density to possible age-related changes in cerebral volume. The barrel is a representation of a single vibrissa in rodent somatosensory cortex [Woolsey and van der Loos, 1970; Woolsey, 1978 (for review)], a morphological and functional subunit whose discrete nature makes it suitable for quantitative studies of cortical aging in an animal model. Thus, cells in one identified barrel, C3, are being counted in coded tissue from male C57B16 mice aged 4 to 33 months [Curcio, 1980].

Barrels as cytoarchitectonic phenomena are most easily seen in thick sections tangential to the pial surface viewed at low power. In contrast, individual cell types are best resolved in thin sections viewed at high power. Therefore, a two-stage approach has been adopted in which barrels identified in thick sections are subsequently re-sectioned for cell counts. Tangential 100 micrometer Vibratome sections through mixed aldehyde-fixed hemispheres are osmicated, dehydrated, and flat-embedded in plastic. Barrels are visible in these unstained preparations, and are photographed and mapped at low power (see Figure 1). Barrel C3 is then re-sectioned at 1 micrometer for cell counts and is examined at high power.

(Figure 1)

Several problems arise in the implementation of this process. First, it is difficult to determine on a cell by cell basis whether or not a cell belongs to a given barrel, particularly at high power. Thus, we need a simple and unbiased method by which to make this decision.

Second, evidence from a number of investigators [White, 1976; Woolsey, 1978] indicates that the cell-rich exterior ring of the barrel ("side") and the cell-poor interior ("hollow") exhibit differential distribution of tissue elements such as cell bodies, axons, and dendrites, which presumably reflects different functions of these two zones. Therefore, analysis of cell density would be more meaningful if the barrel could be subdivided into its constituent zones [Pasternak and Woolsey, 1975]. These two problems might be loosely defined as mapping considerations; i.e., is a cell in a barrel and, if so, where in that barrel.

Third, as has been extensively described elsewhere [Ebbesson and Tang, 1965; Konigsmark, 1970; Weibel and Bolender, 1973; Underwood, 1968], cell counts are affected by the size and shape of the cells being counted and the section thickness. Several methods exist for correcting raw counts into true counts, and all require an accurate measurement of the size of the particle counted. Our use of thin sections demands accurate planimetric data with which to perform these corrections.

Finally, in the interest of developing the barrel as a more complete model for aging research, glial and vascular profiles are included in the analysis. Thus, multiple profile classes, as opposed to a single stereotyped class, are required.

System Goals

To these ends we have developed a computer modeling and graphics system which integrates data from a single barrel at different stages of histological processing and at different viewing magnifications. The barrel boundary seen in thick

sections and profiles of cellular nuclei seen in thin sections are traced onto a digitizing tablet. The computer system then superimposes the barrel boundary on the cell map using landmarks common to thick and thin sections (blood vessels) and determines cell density within the barrel.

The next section of the paper describes the general features of our system. This description is followed by a discussion of our specific implementation.

General Features of the System

Data Acquisition

Barrels in thick sections, nuclear profiles in selected thin sections (see above), and landmarks common to both, are photographed and printed. These two-dimensional data are entered into a computer by tracing from the photographs on an X-Y digitizing tablet. Other information, such as depth of section and feature labels are also entered at this time. The following classes of profiles are entered: barrel, neuron, astroglia, oligodendroglia, microglia, and blood vessel. Features which cannot be unambiguously classified are nonetheless traced and labeled "unclassified." In addition, this program accepts landmarks identified and labeled by the user.

These data are organized in a three-dimensional model, where the Z axis represents the section depth. The model is stored as a text-file, organized by section and, within sections, by profile. Stored in this file is depth, or Z coordinate, as designated by the user, the number of profiles, the number of points per profile, and the set of individual X-Y points describing each profile.

Multiple Drawings

All coordinate values are stated in terms of the actual size of the tissue. This seemingly minor decision greatly influences the design of the rest of our system. It requires that the scale of each section must be known accurately when it is traced. We allow tracings at any scale, but require that a line of known length be entered for each tracing. The use of real world units greatly simplifies subsequent processing.

Alignment of serial or sequential sections is accomplished by the use of landmarks which are defined in each of the fields to be superimposed. Some of these landmarks are actually features in the images (e.g., centers of radial blood vessels). These serve to align serial sections. A related problem arises because the photographs which are traced actually form a mosaic which is too large to trace in its entirety. In this case artificial landmarks are created.

The following approach assumes that the transformation from one drawing to another is a rigid body transformation, involving only rotation and translation in the plane of section. It does not correct for arbitrary rubber-sheeting caused by tissue compression or differential shrinkage during the re-sectioning of the thick sections. Such corrections could be performed [Broccini and Marino, 1980], if necessary, but preliminary results indicated that the rigid body assumption is justified, and that a uniform scaling step may also be required (see below).

Figure 2 illustrates the method used to align drawings. Assume there are two landmarks A and B in drawing 1, and the same two landmarks A' and B' in drawing 2. To superimpose A and B on A' and B', consider the following procedure:

a) translate A to the origin,

b) rotate about the origin until the line segment A-B is parallel to A'-B',

c) translate the origin to A'.

(Figure 2)

This procedure guarantees that A and A' are now superimposed, and that the line A-B is co-linear with the line A'-B'. It does not necessarily superimpose B and B'. However, if all assumptions have been satisfied, then B and B' should be superimposed. Similarly, any other landmark (seen as C and C' in the two drawings) should now be superimposed. The differences in the final positions of like-labeled landmarks serves as a good indicator of the errors introduced by the multiple drawing and transformation procedure.

Although the coordinate systems we are dealing with here are two-dimensional, it is convenient to use a space with one more dimension [see Newman and Sproull, 1973]. The transformations we need (Translation and Rotation) are easy to compute from the positions of A, B, A' and B'. Each such transformation may be represented by a 3x3 matrix. In order to carry out the procedure outlined above, we could apply the individual transformations in the order described above. However, it is also possible to represent the entire sequence of transformations by a single 3x3 matrix. This rather more complex transformation could be solved for analytically, or it can be derived numerically. In either case, the final transformation matrix is the matrix product of the individual transformation matrices. Our system derives the final transformation matrix numerically.

The required translations and rotation are applied to every point in one of the tracings to transform it to the coordinate system of the other tracing. Thus, if cellular profiles traced from the region of C3 and two large blood vessels are in one tracing, and the boundary of C3 traced at low power, along with the same two large vessels, is in another tracing, then the final picture will contain the barrel, the cells, and the landmark vessels--all in the same coordinate system.

Our preliminary results show that this method is adequate for combining drawings which represent different parts of the same section, but that the re-sectioning process produces thin sections which apparently vary uniformly in linear size by as much as 3%. In order to properly align several thin sections and the low-power, thick section from which they came, we scale after step (b) above so that the distances between landmark pairs A-B and A'-B' are identical. This ensures that landmarks B and B' will be exactly superimposed after the translation in step (c).

Display

The three-dimensional model produced above is suitable for display on various graphics devices. Various views may be generated by scaling, translating, or rotating the data in three-dimensions, using 4x4 transformation matrices similar to those described above. These displays range from simple line drawings of the original profiles to color coded, selective display of particular features of interest.

Analysis

Once in the computer, the model may be subjected to several levels of analysis. Parameters calculated for each profile are area, perimeter, major and minor axes and their orientations according to a modified dot-product algorithm [Sloan, 1980], geometric center, and relationship of the center to the nearest portion of the barrel boundary. This relationship is expressed as a distance whose sign indicates if the profile is inside the boundary (positive) or outside (negative). Profiles located "outside" the barrel boundary as defined in the low power map may be discarded. Thus, the decision to include a profile in the sample is made on the basis of an unbiased geometric consideration. The distance parameter also allows calculation of cell densities as a function of location within the barrel, so that properties of side and hollow may ultimately be determined separately.

Cell Counting

The analysis phase produces a distribution of profile counts as a function of profile area. The cell counting program applies two corrections to the raw profile counts according to the procedure of [Coupland, 1968]. First, the profile distribution is transformed to a nuclear size distribution, assuming that nuclei are spherical. The largest observed profiles are assumed to be equatorial transections of the largest particle class. Under this assumption, it is possible to estimate the number of actual particles of this size present. Also, the distribution of minor transections of these large particles can be calculated and subtracted from the observed profile distribution, yielding a distribution of profiles derived from smaller particles. These steps are iterated for each size class until all observed profiles have been accounted for, and a true particle size distribution has been generated.

Secondly, the volume containing a known number of cells must be corrected to yield true density. The total density is calculated by summing across size classes the number of cells in each class divided by the volume in which the centers of those cells were distributed. This effective sampling volume for each particle size class i is

$$V = A \times [t + d(i)]$$

where A is the area sampled (cross-sectional area of the barrel), t is the section thickness, and d(i) is the mean diameter of nuclei in class i. Programs performing similar corrections have been previously published [Anker and Cragg, 1975; Banks et al., 1977; Miller, 1979].

Specific Implementation

Prototype

A prototype system performing the above tasks has been implemented. The prototype system's hardware consists of a Tektronix digitizing tablet and terminal connected to a DEC-10 computer, a large time-sharing machine in the Medical Center Computing Facility (MCCF) at the University of Rochester. This hardware was available for general use, as a service of the MCCF. Therefore, no initial hardware investment was required.

The software for this prototype system has been written in FORTRAN on the MCCF DEC-10. The data acquisition program, NEURO, accepts profiles and landmarks labeled by the user and a reference line of known length in order to transform the coordinates produced by the digitizing tablet to an "actual-size" coordinate system. NEURO produces a single drawing file for every "tablet-sized" drawing.

A second program, MERGE, combines two files created by NEURO into a third drawing file. MERGE serves to concatenate data from drawings too large to fit completely on the small digitizing surface of the tablet and to integrate several drawings of the same area (drawn at different magnifications, for example). The files created by NEURO, and accepted by MERGE, have two major sub-divisions. The first part of the file is devoted to a listing of the landmark points. Each landmark point has an identifier, and an X-Y position in the local coordinate system. MERGE reads the two lists of landmarks, and searches for landmarks which appear in both drawings. These common landmarks are the key to the alignment procedure. From a list of common landmarks, MERGE chooses the two distinct landmarks which are farthest apart. This choice tends to improve the precision of subsequent calculations. This pair of common landmarks is used to create a transformation from one of the coordinate systems to the other (the choice here is arbitrary). Once the transformation has been calculated, it is applied to all of the data points (including the landmarks) of the file which is to be transformed. Note that a particular landmark may now be represented by more than one set of X-Y coordinates. The distance between these multiple representations of the "same" point serve as an indicator of the errors introduced by the digitization and alignment processes.

Several display programs have been written. One is a FORTRAN program which displays the model on a Tektronix terminal. A similar program has been written in SAIL to take advantage of a full-color raster graphics system located in the Computer Science Department. On this display, we can selectively color (or make invisible) any subset of the profile classes (Fig. 3), color code the serial sections, or show the 3-D model rotating in real time [Sloan and Brown, 1979].

(Figure 3)

Local analysis of the resulting model is performed by CELL, which reports for each profile its class, area, radius, perimeter, geometric center, orientation, major and minor axes, and distance from the barrel wall. This information is stored in a summary file, for further analysis.

The cell-counting program is an implementation of Coupland's procedure which reads the summary file produced by CELL and selects only profiles in the class of interest (e.g., neurons) whose mapping parameters indicate they are inside the barrel boundary. From a profile area distribution is generated a particle diameter distribution, which is then corrected to yield a density distribution. The output file of this program contains raw and corrected distributions and may be plotted in histogram form using a standard Tektronix graphics package (see Fig. 4).

(Figure 4)

Laboratory Version

The data acquisition process is not well suited for a heavily used central time-sharing machine. All of the functions provided by NEURO can be performed on a much smaller, personal machine. We have re-written this part of the system in PASCAL on an APPLE II PLUS micro-computer, which is equipped with sufficient local storage (64K plus two mini-floppy disk drives) to temporarily hold several drawings. We now do data acquisition on the small machine, in the laboratory, and automatically transfer the drawing files by way of a telephone line interface to the MCCF DEC-10 for the analysis described above. The hardware for the laboratory version was assembled from off-the-shelf components for under $6,000. This arrangement has the advantage that the small machine is under the complete control of the investigator when the drawings are digitized, a process which demands considerable concentration.

In general, it is appropriate to use a personal micro-computer such as the APPLE II for jobs which require considerable customizing and friendly user interaction. In moving our data acquisition program to the small machine, we have been able to add several features which would have been difficult, or expensive, to provide on a large time-shared computer. For example, in the prototype system, the user was required to type certain information on a keyboard. Some of this typing has been replaced by pointing at a "menu" on the digitizing tablet.

Another problem with the prototype system involved the choice of digitizing precision. In general, the digitizing tablets we have used provide more than enough precision. Too much precision is a problem in our system because we want to save the entire digitized profiles for later analysis. In the customized laboratory version we are able to use the micro-computer to filter out points in the profiles, discarding points which are "close to" the previous point [Cowan and Wann, 1973]. This process would be slow and expensive on the large machine, but comes for free on the micro-computer. In fact, the current system offers a choice of digitizing precisions (fine, medium, and coarse), which are menu-selectable.

It is extremely useful for the person tracing cell profiles to be able to see a display of the tracing as it happens. In the prototype system, this display was primarily used to detect gross errors and to reject individual profiles when such errors occurred. The micro-based system has allowed us to add more sophisticated graphical editing, such as the capability to erase individual points or whole profiles from the viewing screen. Again, these features could have been provided in the prototype system, but the cost and effort would have been much higher.

It is also possible to do some small amount of local analysis on the micro-computer; however, the topographical analysis performed in our current work requires access to the facilities available only in a fairly large computation center. Similarly, the display and statistical analysis packages which are typically available at a centralized computation center would be expensive to duplicate effectively. Sophisticated statistical analysis packages reflect a great deal of software development and require somewhat more computational power than that available from a micro-computer. The graphics facilities are largely a matter of hardware investment. The display capabilities on a micro-based system are accessible, but necessarily limited in quality. A central computer center can afford to invest in high-quality display devices.

Our laboratory micro-computer has enough local storage to allow an investigator to trace drawings in so-called "stand-alone" fashion. That is, the micro-computer is not connected to the central time-sharing machine during the data acquisition phase. Drawing files are created and stored on mini-floppy disks.

The transfer of these drawing files to the main-frame computer is an entirely automatic process. The micro-computer is equipped with a telephone interface which allows it to place phone calls under program control. Most time-sharing computer centers have "dial-in" telephone lines for remote terminals. It is very easy to write a program for the micro-computer which will call the computer center and act as if it were a terminal.

In order to transfer files, the micro-computer calls the main-frame machine at a time selected by the user, logs in as a human user would, and starts a companion program running on the big machine. The two programs then effect the file transfer, checking for errors and re-transmitting information as needed. We use a "packet-based" protocol for the conversation between these two programs. When the micro-computer is satisfied that all files have been successfully transferred, it terminates the companion program, logs off the main-frame machine, and hangs up the phone.

Typically, this entire process takes place at night, when the rates are low at the computer center. The program will detect all errors and try to correct them; sometimes taking the extreme measure of hanging up the phone and trying again.

Discussion

A major concern in our concrete problem is the mapping parameter, distance from barrel boundary, which allows selection of profiles as a function of position. Other investigators designing similar systems might be interested in mapping parameters better suited for their own needs. For example, one might wish to study profiles as a function of distance from a central point, as in the retina, distance along an A-P or other axis, as in an analysis of projection neurons in a nucleus [Loughlin et al., 1979], or location in a radial sector, as in peripheral nerve [Dunn et al., 1976]. We believe that flexibility in choosing the particular features to be measured and the type of analysis to be done is best maintained by saving all of the x,y points defining the individual profiles, and only later reducing these data to summary form.

One of the potential applications of our system is stereological analysis. Stereology is a method whereby inferences may be made about three-dimensional structures from randomly chosen slices (histological preparations) through a tissue block [Weibel and Bolander, 1973]. In most stereological formulations a test grid is placed over a field of view, and the number of intersections of the grid with the feature of interest is used as an estimate of number, length, or cross-sectional area of that feature, which may be extrapolated into the third dimension. Following the local analysis of the raw data described above, these parameters for a given class of profiles are known directly. Furthermore, since locations of profiles are saved, such analyses may be done with respect to position; for example, volume fraction of capillaries in the barrel wall may be determined.

In our system, the task of the operator is to accurately trace and identify features of interest. Many of these identifications require the judgment of a highly trained observer. However, almost all of the information that such an observer can extract from a field of view is well represented by the labeled profiles in our raw data files. Once these data have been made machine readable, there are an almost limitless number of methods of analysis which can best be done by the computer, or in which computer assistance (e.g., display) is very helpful. Our goal has been to find the right division of labor between trained observer and computational analysis.

Acknowledgments

We thank Drs. P.D. Coleman and R.M. Herndon for support and encouragement, and S. Fram for programming assistance. The preparation of this paper was supported in part by N.I.H. Training Grant GM07136, N.I.H. Grant AG1121, N.I.H. Grant NS15283, and the Defense Advanced Research Projects Agency, monitored by the ONR, under Contract N00014-78-C-0164.

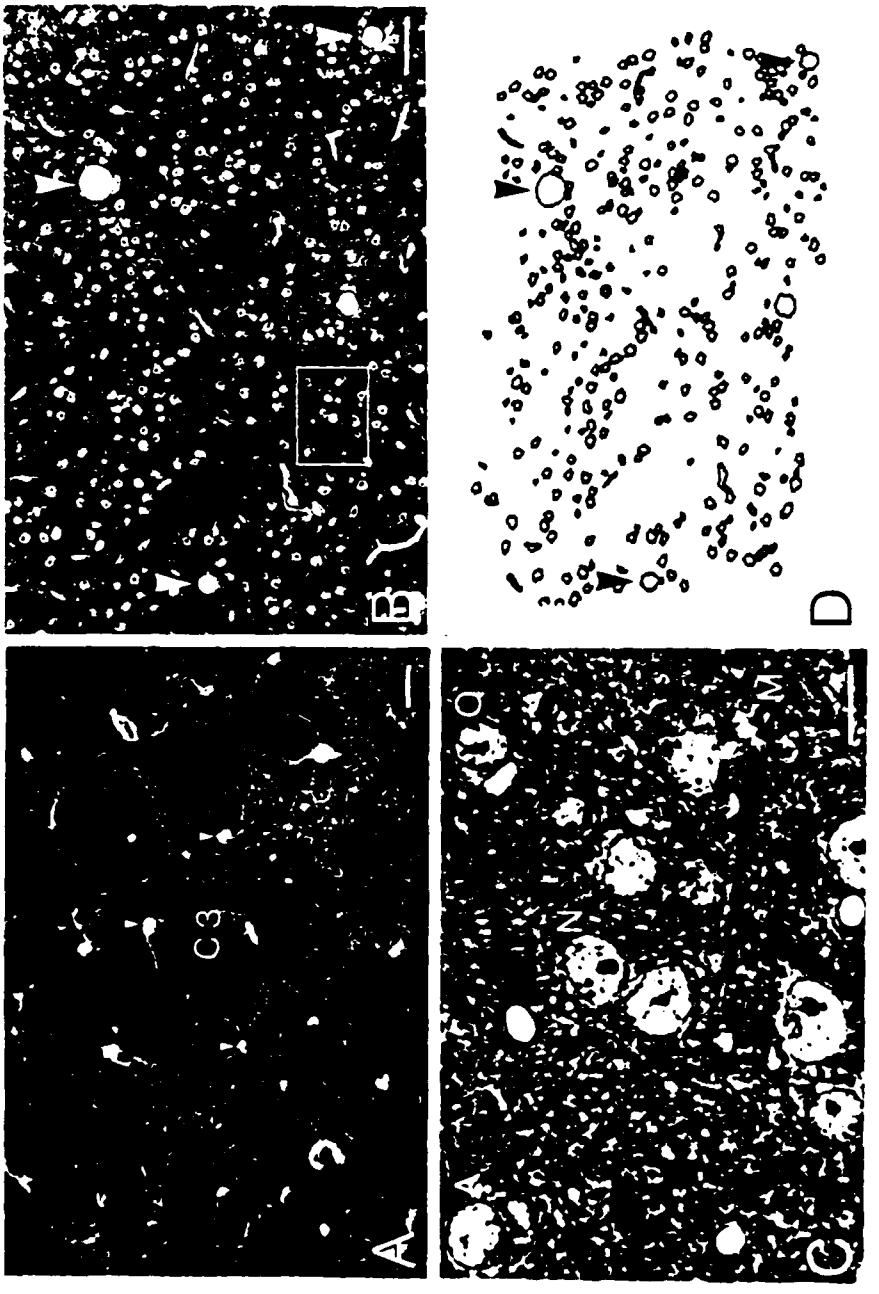
Figure Legends

Figure 1. Thick and thin tangential sections through mouse somatosensory cortex. A) Barrels in thick unstained preparations. Cell nuclei appear as pale ghosts, and the neuropil appears dark; thus, the cell-rich walls are lighter than the neuropil-rich hollows. Barrel C3 and landmark vessels used for alignment of sections are indicated (arrowheads). Mixed aldehyde-fixation, 100 micrometer Vibratome section, osmicated, and plastic-embedded. Bar = 100 micrometers. B) 1 micrometer section obtained from thick section in (A) with same landmark vessels indicated (arrowheads). Stevenel blue stain [del Cerro et al., 1980]. Box is field shown in (C). Bar = 50 micrometers. C) Box in (B) at higher power showing cells in barrel wall. N = neuron, A = astrocyte, O = oligodendrocyte, M = microglial cell. Bar = 10 micrometers. D) Map of section in (B) displayed by computer with landmark vessels (arrowheads). All neuronal and glial nuclear and vascular profiles seen in region of C3 were traced onto the digitizing tablet and displayed. (See text for details of data acquisition and display processes.)

Figure 2. Transformation of two maps with common landmarks into unified coordinate system. The problem is to transform the coordinate system of I to that of IV. We proceed by Translating so that landmark A is at the origin (II), then Rotating until A-B is parallel with A'-B' (III), and finally Translating the origin to A' (IV). A and A' now coincide exactly.

Figure 3. Computer displays of data from four thin sections, including the one shown in Fig. 1B-D, spaced twenty micrometers apart, and the low power barrel map after alignment in the Z direction. A) All cell and vascular profiles seen in four 1-micrometer sections with outline of barrel, seen in low power map, superimposed. B) Display of only neuronal nuclei from same four sections, with barrel added. (See text for details of selective display process.) Note the central area of lower cell density and string of neurons in side of adjacent barrel (arrows). C) Blood vessels. Vessels indicated by arrowheads are those seen in Figure 1 and were used for alignment of sections. D) Glial cells only.

Figure 4. Upper left: Size distribution of profiles of neuronal nuclei from case displayed in Figs. 1 and 3. Lower left: Corrected particle size distribution generated by the procedure of Coupland (see text for details). Upper right: Density distribution created by correcting particle size distribution for effective sampling volume. Lower right: Total profiles, particles, and density figures obtained by collapsing histograms.



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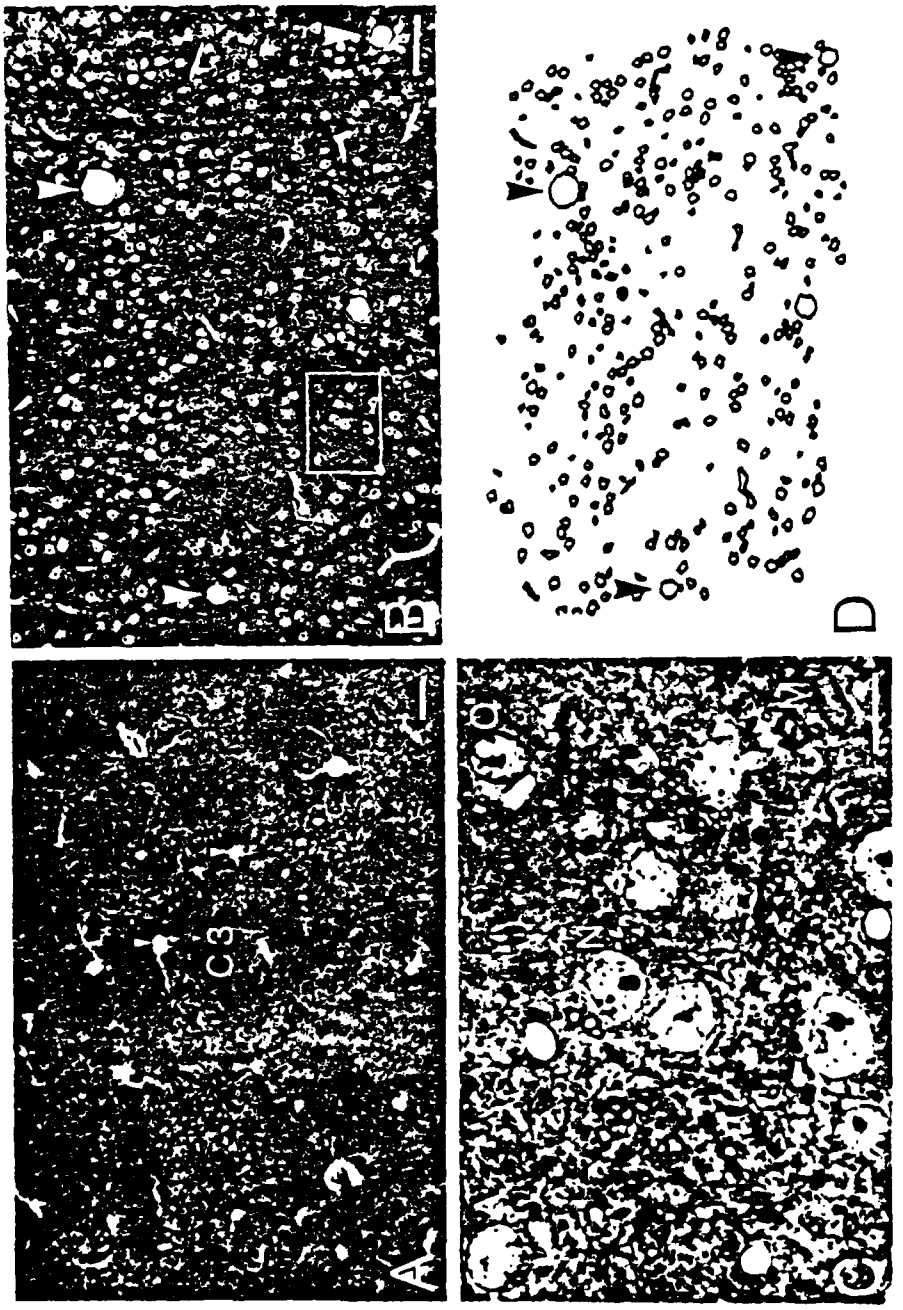

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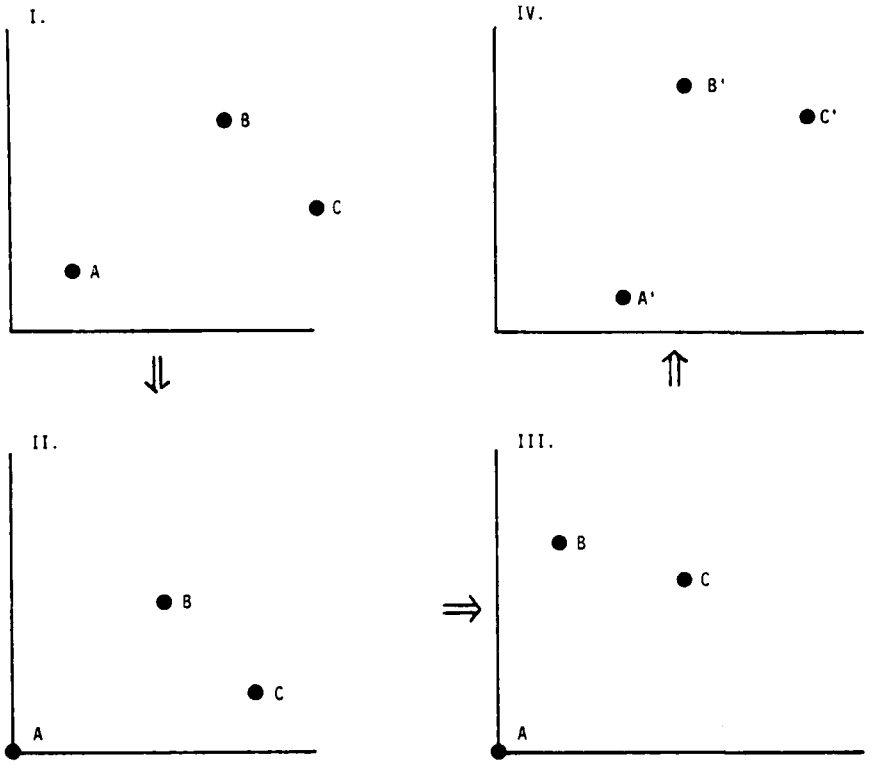

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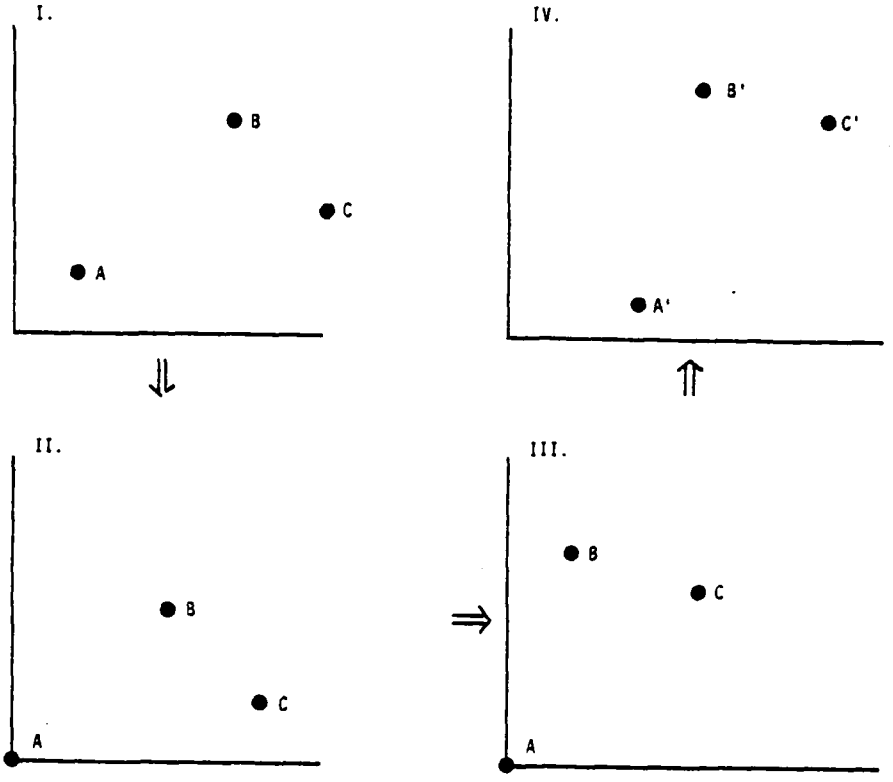

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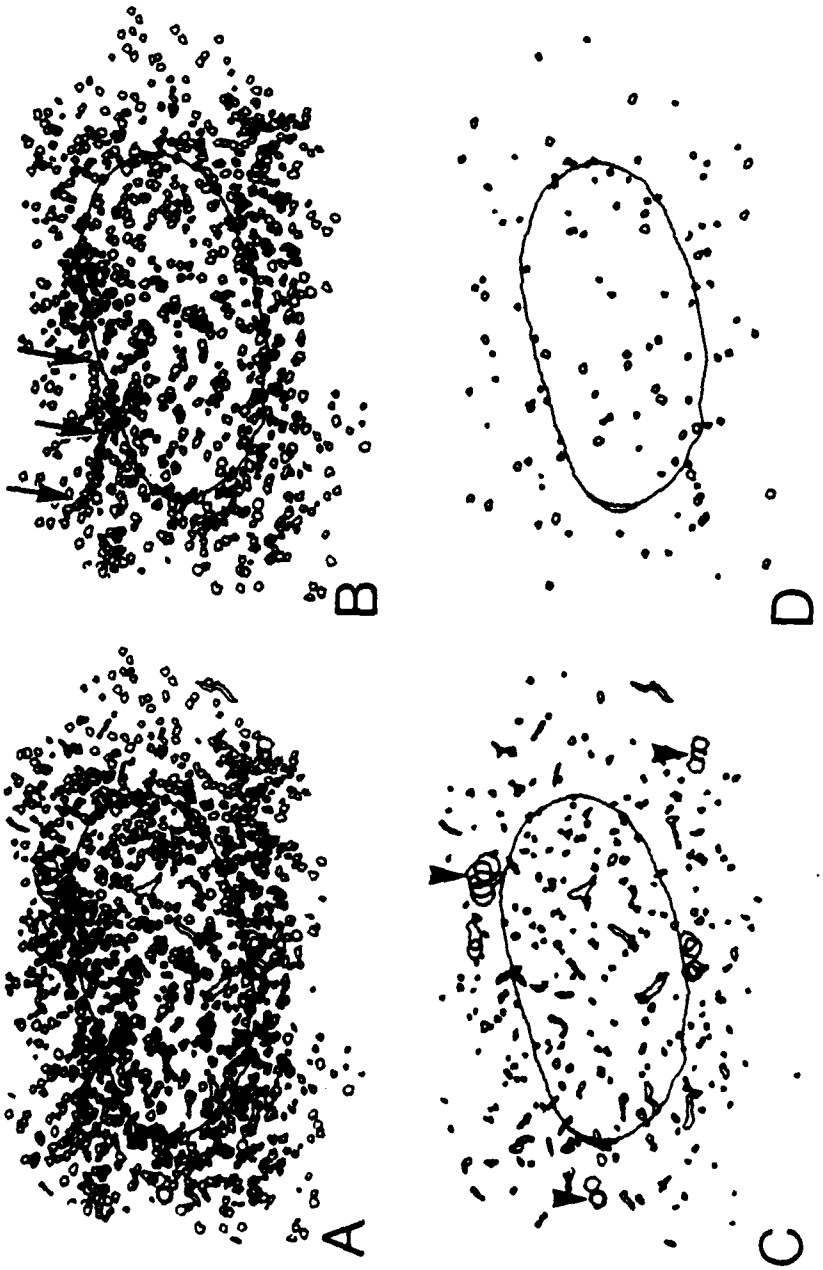

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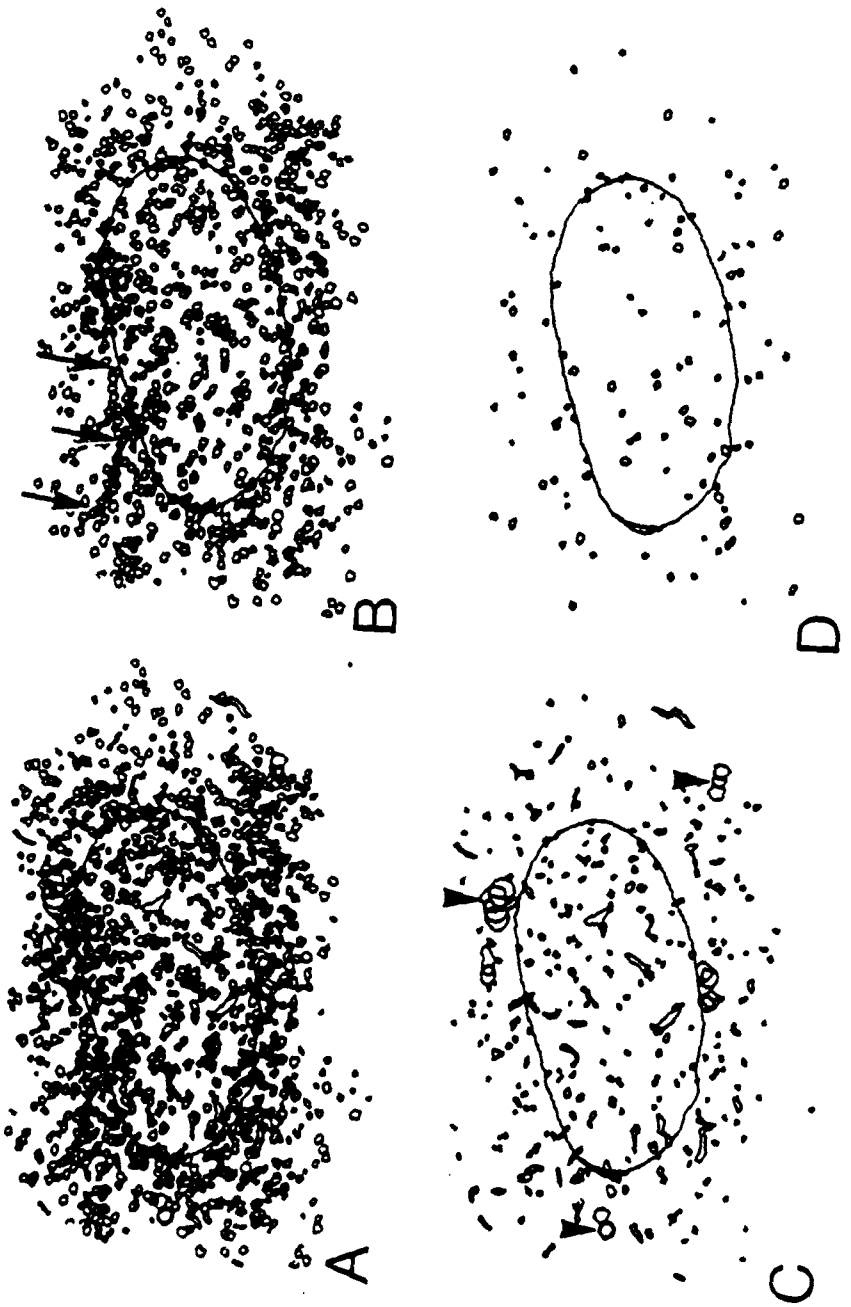

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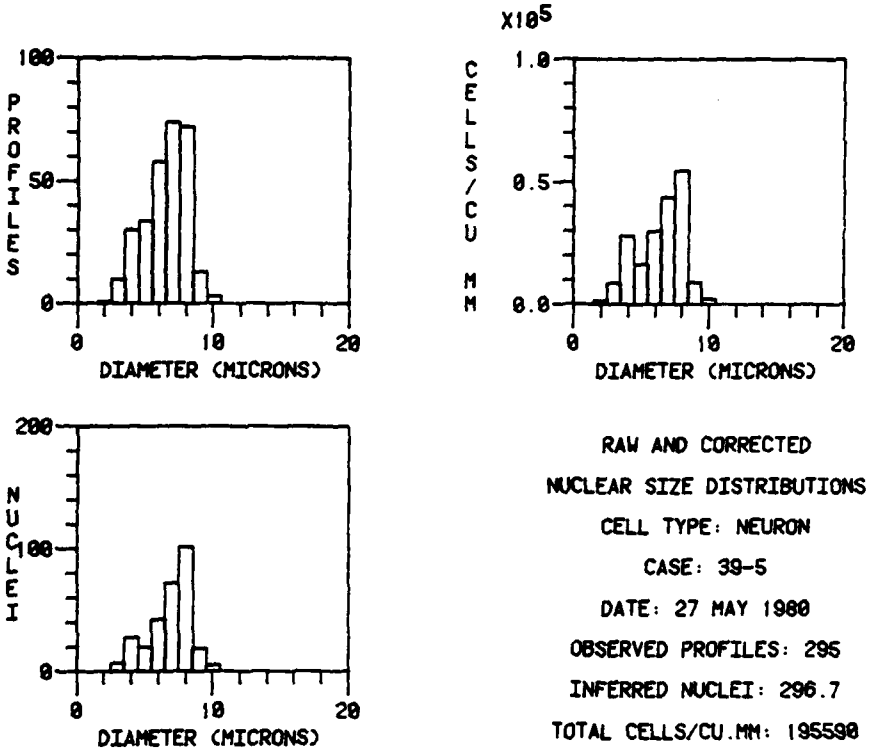
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Figure 4.

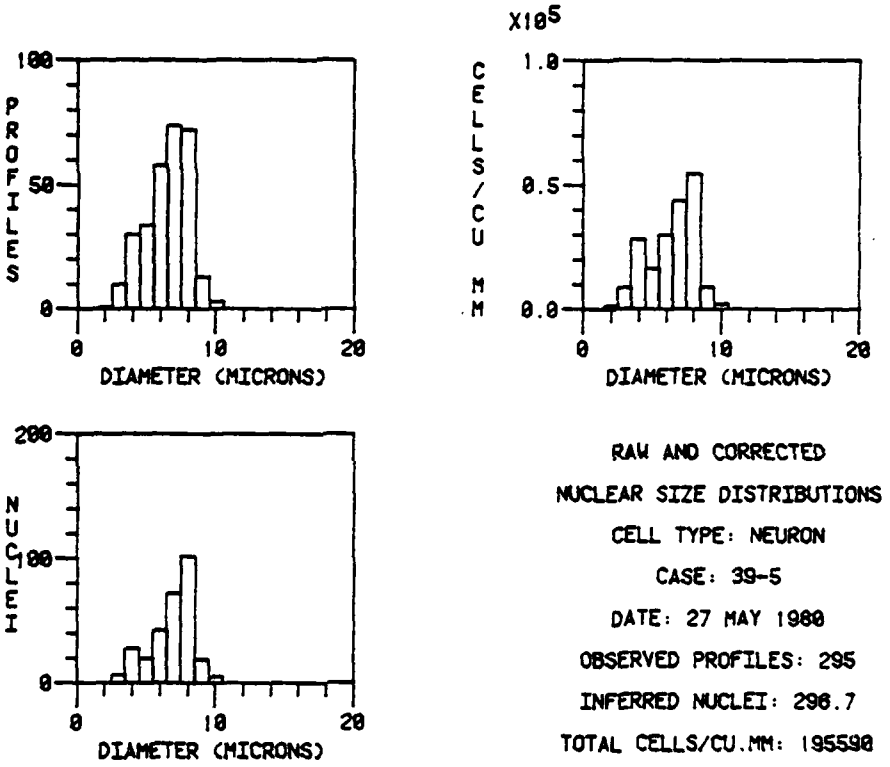
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Figure 4.

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